



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

April 25, 2014

MEMORANDUM

Subject: Protocol Review for 90435PA1 (Virucidal Efficacy of a Disinfectant/Efficacy of a Disinfectant or Sanitizer Applied to a Room Via a Fogging, Misting, or Vaporizing Device); DB Barcode: D417767.

From: Ibrahim Laniyan, Ph.D.
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A handwritten signature in blue ink, likely belonging to Ibrahim Laniyan, is written over the "From:" field.

Thru: Mark Perry
Team Leader
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Handwritten initials "MP" in black ink are written next to the "Thru:" field.

To: Demson Fuller, RM 32
Regulatory Management Branch II
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Applicant: ATS Labs
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I. BACKGROUND

ATS Labs intends to test efficacy of antimicrobial products bearing disinfection and sanitization claims of enclosures via fogging and misting application. Through the current submission, the registrant is submitting two protocols proposing virucidal disinfection, disinfection or sanitization of hard non-porous surfaces present in visibly clean room treated via a fogging, misting, or vaporizing device. Protocols were developed by ATS Labs, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

This data package is identified as D417767 contained a letter from the applicant's representative (dated November 11, 2013), four studies (MRID nos. 492659-01, 492626-01, 493679-01, and 493679-02) but only two to be reviewed.

II. BRIEF DESCRIPTION OF THE PROTOCOL

Note: During the course of the protocol review, the version of the protocol with MRID 492659-01 and MRID 49262601 have be replaced with the latest version MRID 493679-01 and MRID 493679-02 (Version rev.2, dated April 23, 2014). The following is the review of MRID 493679-01 and MRID 493679-02.

1. MRID 493679-01 “Efficacy of a Disinfectant or Sanitizer Applied to a Room via a Fogging, Misting or Vaporizing Device” by Scott R. Steinagel. ATS Labs Protocol Dated – April 23, 2014.

The purpose of this assay is to evaluate the efficacy of a room disinfection or sanitizing system applied by a fogging, misting or vaporizing device on hard, non-porous surfaces.

Method References:

1. Association of Official Analytical Chemists (AOAC) Official Method 961.02, Germicidal Spray Products as Disinfectants. In Official Methods of Analysis of the AOAC, 2012 Edition.
2. Association of Official Analytical Chemists (AOAC), Official Method 965.12. Tuberculocidal Activity of Disinfectants. In Official Methods of Analysis of the AOAC, 2012 Edition.
3. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
4. Association of Official Analytical Chemists (AOAC) Official Method 955.17, Fungicidal Activity of Disinfectants. In Official Methods of Analysis of the AOAC, 1955 Edition.
5. American Society for Testing and Materials (ASTM). Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporocidal Activities of Liquid Chemical Germicides, E 2197-11.
6. American Society for Testing and Materials (ASTM). Standard Test Method for Production of *Clostridium difficile* Spores for Use in Efficacy Evaluation of Antimicrobial Agents, E 2839-11.
7. American Society for Testing and Materials (ASTM). Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces, E1153-14.
8. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Draft Protocol “Protocol for Sterilization of Porous and Non-Porous Surfaces within Sealed Enclosures using Vaporized Hydrogen Peroxide”.

9. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
10. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2100: Sterilants – Efficacy Data Recommendations, September 4, 2012.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.
12. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2300: Sanitizers for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.

Test System (Microorganism):

- Limited Spectrum Disinfection (Gram positive bacteria): *Staphylococcus aureus* (ATCC 6538)
- Limited Spectrum Disinfection (Gram negative bacteria): *Salmonella enterica* (ATCC 10708)
- Broad Spectrum Disinfection: *Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708)
- Hospital or Healthcare Disinfection: *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442)
- Fungicidal Disinfection: *Trichophyton mentagrophytes* (ATCC 9533)
- Tuberculocidal Disinfection: *Mycobacterium bovis* - BCG
- Disinfection of *C. difficile* spores: *Clostridium difficile* (ATCC 43598)
- Food Contact Surface Sanitization: *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229)
- Non-Food Contact Surface Sanitization: *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048)

Procedure:

A visibly clean enclosure will be used. Depending on the application, this may be represented by an entire room or large chamber. Shelves with supports, laboratory carts or other suitable means of supporting the test carriers may be positioned within the enclosure. The enclosure will be appropriately sealed prior to testing in order to isolate the space in which the test substance will be applied.

For the main bacterial organisms (*S. aureus*, *S. enterica* and *P. aeruginosa*), a minimum of 60 carriers must be evaluated per test organism, per test cycle. If the cubic volume of the room used exceeds 100 m³, the minimum number of test carriers tested will be determined using the following equation:

$$\text{Number of Test carriers} = [(m^3 - 10) / 2] + 15$$

Where m³ = the volume of the room enclosure in cubic meters

For the main bacterial organisms (*S. aureus*, *S. enterica* and *P. aeruginosa*), triplicate carriers at a minimum of twenty designated locations, totaling 60 test carriers per test organism, are required to substantiate efficacy claims. The locations that have been selected are designed to evaluate the extremes of the enclosure. Additional locations may be added to achieve the minimum required number of test carriers.

For all supplemental claims (fungicidal, tuberculocidal, non-food contact sanitization, food-contact sanitization and disinfection of *C. difficile* spores), a single carrier at a minimum of twenty designated locations, totaling 20 test carriers per test organism, are required to substantiate efficacy claims. The locations that have been selected are designed to evaluate the extremes of the enclosure.

These locations are designed to evaluate the following areas of the room:

- All corners
- Central locations on all wall faces
- Central locations on the floor
- Multiple locations and heights
- Underneath horizontal surfaces

The actual volume of the testing enclosure will be determined and reported.

To ensure the minimum concentration of active is delivered to the room, the Sponsor is responsible for providing a method to monitor the level of active ingredient present in the room during each test cycle. Chemical indicators or detection device(s) may be used. At minimum, an indicator or detection device/probe will be placed at a location representing where the concentration of active is expected to be the lowest (worst-case condition). The detected level of active will be reported. If a detection method is not available to detect the specific active ingredient, the Sponsor is responsible for providing justification as to the level of active ingredient applied in each test cycle.

Non-Active Treatment Control

A control will be performed to evaluate the reduction of the test organism after exposure to a Sponsor-provided formulation containing no active ingredient. If such a formulation cannot be provided, sterile deionized water may be used in its place. It is recommended that this control be performed prior to testing to ensure the acceptance criterion can be met. The test cycle parameters (including length of time) must be carefully considered to ensure this control meets the designated acceptance criterion.

In a separate test cycle, the room or enclosure will be prepared in a manner consistent with the test cycles. A total of three inoculated and dried carriers, per test organism, will be evaluated in this control at locations representative of the test cycle. The first carrier will be placed at location #1, the second carrier will be placed at location #7 and the third carrier will be placed at location #15 to represent each orientation at high, mid and low locations around the room. (Refer to figure 1.) For the main bacterial organisms (*S. aureus*, *S. enterica* and *P. aeruginosa*), one carrier will be oriented horizontally with the inoculum facing downward, one carrier will be oriented vertically and one carrier will be oriented horizontally with the inoculum facing upward. For all supplemental claims (fungicidal, tuberculocidal, non-food contact sanitization, food-contact sanitization and disinfection of *C. difficile* spores), each carrier will be oriented horizontally with the inoculum facing upward. These orientations are designed to simulate the test cycle orientations. The actual locations and orientations will be documented and reported.

The carriers will be exposed to the non-active solution in a manner consistent with the intended test cycle parameters.

The acceptance criterion for the non-active treatment control is a minimum log₁₀ value of 3.0 for each test organism.

2. MRID 493679-02 “Virucidal Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device” by Shanen Conway. ATS Labs Protocol Dated – April 23, 2014.

The purpose of this assay is to evaluate the efficacy of a room disinfection system applied by a fogging, misting or vaporizing device on hard, non-porous surfaces.

Method References:

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1053-11.
2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1482-12.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Draft Protocol "Protocol for Sterilization of Porous and Non-Porous Surfaces within Sealed Enclosures using Vaporized Hydrogen Peroxide".
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
5. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations, September 4, 2012.
6. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
7. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.

Test System:

The virus will be obtained from the American Type Culture Collection, Manassas, VA or from an alternate, reputable source.

The appropriate indicator cell line, which supports the growth of the test virus, will be used in this study. The cells may originally be obtained from the American Type Culture Collection, Manassas, VA and propagated by ATS Labs personnel or received from a reputable source.

Procedure:

A visibly clean enclosure will be used. Depending on the application, this may be represented by an entire room or large chamber. Shelves with supports, laboratory carts or other suitable means of supporting the test carriers may be positioned within the enclosure. The enclosure will be appropriately **sealed** prior to testing in order to isolate the space in which the test substance will be applied.

For each batch of test substance, a single carrier will be evaluated in each of the twenty designated locations, yielding a total of 20 test carriers. These locations are designed to evaluate the following areas of the room:

- All corners
- Central locations on all wall faces
- Central locations on the floor
- Multiple locations and heights
- Underneath horizontal surfaces

Success Criteria:

1. All media sterility controls must be negative for growth.
2. The media growth control must be positive for growth.
3. All test microorganisms must demonstrate culture purity.
4. Neutralization is validated as described.
5. Soil sterility control is negative for growth.
6. Dried Virus Recovery Control Film must demonstrate an average $\geq 4\log_{10}$ /carrier for a valid test.
7. Non-Active Treatment Control must demonstrate an average $\geq 3\log_{10}$ /carrier for a valid test.
8. Final when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level for a virucidal disinfection.

III. CONCLUSION AND COMMENTS

1. The submitted protocol (MRID 493679-01) **is adequate for testing** efficacy of a disinfectant or sanitizer on hard non-porous in a room treated via a fogging, misting, or vaporizing device.
2. The submitted protocol (MRID 493679-02) **is adequate for testing** virucidal efficacy of a disinfectant on hard non-porous in a room treated via a fogging, misting, or vaporizing device.
3. It is a reminder that product must be tested at the lower certified limit proposed on the CSF of tested products.
4. The potential variability in the method must be addressed prior to data generation. The Agency encourages the testing laboratory to assess the degree and sources of variability introduced by any significant method modification – this information should be supplied to the Agency prior to GLP testing. For example, preliminary runs of the study should be performed to determine the degree of variability associated with control and treated carriers; the number of carriers should be increased if the variability is too high.
5. Identify and use the most recent versions of all standard methods cited in the protocol. Specify the broth media for generating test cultures and the plating medium for recovery of each test microbe [Use the AOAC Use-dilution method for preparation of cultures of *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), or *Staphylococcus aureus* (ATCC 6538).]
6. The study controls must perform according to the criteria detailed in the protocol. If any of the control acceptance criteria are not met, the test may be repeated.
7. Provide a list of all deviations or modifications to a standard method.
8. In the disinfectant and sanitization protocol, the neutralization confirmation method for *C. difficile* involves the addition of a small number of spores directly to the filter. This step should be replaced with a step that adds the inoculum to the neutralizer/product mixture and should be followed by a step that allows filtering after a period of several minutes.